

This Page Is Inserted by IFW Operations  
and is not a part of the Official Record

## **BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning documents *will not* correct images,  
please do not report the images to the  
Image Problems Mailbox.**





## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>4</sup> :</b>  <b>C12M 3/02</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 86/ 00636</b>  <b>(43) International Publication Date:</b> 30 January 1986 (30.01.86)
<b>(21) International Application Number:</b> PCT/GB85/00307 <b>(22) International Filing Date:</b> 9 July 1985 (09.07.85) <b>(31) Priority Application Number:</b> 8417590 <b>(32) Priority Date:</b> 10 July 1984 (10.07.84) <b>(33) Priority Country:</b> GB  <b>(71)(72) Applicants and Inventors:</b> STARKIE, Selby, John [GB/GB]; 8 Weston Grove, Gulbourn, Cambridge, CB1 5DY (GB). HOPKINSON, David, George [GB/GB]; The Village Stores, Faulkland, Somerset, BA3 5UX (GB).  <b>(74) Agent:</b> GILL JENNINGS & EVERY; 53/64 Chancery Lane, London, WC2A 1HN (GB).		<b>(81) Designated States:</b> AT (European patent), BE (European patent), CH (European patent), DE (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent), US.  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> CELL GROWTH		
<b>(57) Abstract</b>  <p>A method for the growth of cells in a nutrient medium, which comprises causing the nutrient medium to flow upwardly with decreasing velocity, substantially without turbulent flow, and allowing cells to grow at an equilibrium level in the flow. The method can be conducted in apparatus comprising a vessel (1) of increasing and then decreasing cross-section (greatest at 2) in the direction of flow from a bottom inlet (3) to a top outlet (4). The used medium can be regenerated, if desired.</p> <div data-bbox="958 1239 1331 1932" data-label="Diagram"> <p>The diagram shows a vertical vessel (1) with a bulbous middle section. At the bottom, there is an inlet (3) connected to a pump (P). The vessel widens to a maximum cross-section at point 2 and then narrows towards the top outlet (4). A dashed horizontal line at level 5 indicates the equilibrium level where cells grow. Other labels include 1 for the vessel body, 4 for the top outlet, and 6, 7 for internal features or flow paths.</p> </div>		

***FOR THE PURPOSES OF INFORMATION ONLY***

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GA	Gabon	MR	Mauritania
AU	Australia	GB	United Kingdom	MW	Malawi
BB	Barbados	HU	Hungary	NL	Netherlands
BE	Belgium	IT	Italy	NO	Norway
BG	Bulgaria	JP	Japan	RO	Romania
BR	Brazil	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	LI	Liechtenstein	SN	Senegal
CH	Switzerland	LK	Sri Lanka	SU	Soviet Union
CM	Cameroon	LU	Luxembourg	TD	Chad
DE	Germany, Federal Republic of	MC	Monaco	TG	Togo
DK	Denmark	MG	Madagascar	US	United States of America
FI	Finland	ML	Mali		
FR	France				

-1-

CELL GROWTHFIELD OF THE INVENTION

This invention relates to a method for cell growth, and in particular to the growth of cells in a nutrient medium.

5 BACKGROUND OF THE INVENTION

It is of course conventional to grow cells in a nutrient medium. The nutrient medium may be stationary, e.g., in a dish, in which case it is tiresome, especially on a large scale, to separate the products of cell growth  
10 from the system. Cells may also be grown in a fluent medium, e.g. in order to stimulate cell/nutrient contact and in order to facilitate the replacement of nutrient as it becomes exhausted; however, it is usually then necessary to stir the system, and stirring involves  
15 undesirable mechanical attrition.

PRIOR ART

GB-A-1546554 describes maintaining cells, e.g. lymphoblastoid cells, in suspension in a nutrient medium. As the result of introducing oxygen into the bottom of a  
20 cylindrical channel containing the medium, the medium flows upwardly at a uniform rate. The medium reaching the top of the channel is passed downwardly, outside the channel, to provide circulatory flow. Accordingly, cells are maintained in suspension by careful choice of oxygen  
25 inflow rate.

Examples of documents in which cells are stirred or otherwise moved in a nutrient medium are WO-A-84/03709, in which there is a circulatory flow and an orthogonal flow component caused by the introduction of oxygen;  
30 EP-A-0078061, in which the partial pressure of oxygen is changed during the growth phase (the cells must be non-adhering mammalian cells); and WO-A-82/03227, in which laminar flow is maintained in a "movable culture unit".

35

-2-

EP-A-0050562 states as its object a process according to earlier proposals for cell growth, but in the complete absence of any means of cell immobilisation. The disclosure is of circulating a nutrient medium, in sequence, downwardly through two cylindrical vessels, into one of which oxygen and nutrients can be introduced. The other is the "culture vessel", which includes towards its lower end means for separation of tissue material, allowing the downward passage of medium for recirculation.

#### SUMMARY OF THE INVENTION

A method for the growth of cells in a nutrient medium comprises causing the nutrient medium to flow upwardly with decreasing velocity, substantially without turbulent flow, and allowing cells to grow at an equilibrium level, in the flow. The invention is based upon the principle of flotation, so that cells of one type can grow at a given level in a steady flow, according to the upward velocity of the nutrient medium and the opposing force of gravity, but important advantages are associated with this system.

#### ADVANTAGES OF THE INVENTION

Firstly, apparatus can be engineered to provide a wide variation of flow velocities which can allow the simultaneous growth of various cells (which can form different strata, according to their sedimentation velocity, in the apparatus). Secondly, cells grow very well in such a system, supported only by the medium, and indeed faster than in a stirred system where they are caused to move. Thirdly, it can easily be arranged that the cells are constantly provided with fresh medium, e.g. if spent medium removed from the top of the apparatus is regenerated or replaced before being passed back up the apparatus. Fourthly, and perhaps most importantly, the products of cell growth, metabolism or lysis are passed

-3-

upwardly through the apparatus and can be separated from the medium in a cell-free environment; the products may be, for example, antibodies, other desirable proteins and also dead matter, e.g. from cell walls. Cells may die naturally or may be induced to rupture by the introduction of suitable materials into the flow, e.g. cell wall-destroying enzymes.

#### BRIEF DESCRIPTION OF THE DRAWINGS

The invention will now be illustrated by way of example only with respect to the accompanying drawings, in which:

Figure 1 is an essentially schematic view of apparatus which can be used in the method of the invention;

Figures 2, 3 and 5 are scale diagrams of three further alternative apparatus which can be used in the invention;

Figures 4 and 6 illustrate systems in which the invention can be operated, the latter in the form of a flow diagram; and

Figure 7 is a schematic representation of a unit which can be used as part of the system illustrated in Fig. 6, and in more detail than Fig. 6.

#### DETAILED DESCRIPTION OF THE INVENTION

Figs. 1 to 5 show a vessel 1 having a section of gradually increasing and then decreasing diameter, the maximum diameter being shown at 2. There is an inlet 3 at the lower end of the vessel and an outlet 4 at the upper end. The outlet 4 can lead, for example, to means for collection and/or regeneration and/or separation.

Fig. 1 also shows a pump P. In operation, the pump P causes nutrient medium to flow upwardly through the vessel 1. Whatever means is used to cause flow, in any illustrated apparatus, the flow velocity is greatest at the inlet 3 and the outlet 4, and least at the greatest

-4-

diameter 2. There is a continuous variation in flow velocity. Cellular material which has been introduced into the vessel finds a certain level, and three stratified bands of cells of different densities are shown at 5, 6 and 7. Products of cellular degradation and metabolism are obtained through the outlet, and the design of the apparatus ensures that there is no turbulent flow below 2, i.e. at the levels of the bands at least. No strata can be formed above the greatest diameter 2, where flow accelerates and removes all material from the vessel 1.

Additional features illustrated in Figs. 2 to 5 are arrows indicating the flow of direction of the medium; a reservoir intake point 8 and a medium collection unit 9 (partially illustrated) in Fig. 2; a reservoir 9 including a Marriott tube 10, silicone tubing 11 and a filling bell 12 in Fig. 4; and three sampling tubes 13 in Fig. 5. The design of the apparatus illustrated in Fig. 4 is adapted to the use of higher flow rates than can be used in other illustrated apparatus, without turbulence. Relatively high flow rates may be useful when, as is the case for lymphoblastoid cells, clumps of cells can form which can be maintained in suspension (obviously relatively nearer the bottom of the vessel than individual cells). The apparatus of Fig. 4 provides a trap to prevent back-flow of adherent cultured cells into the reservoir. The tube from the culture vessel to the filling bell prevents the accumulation of bubbles of gas absorbed through the silicon tubing.

Fig. 6 shows a culture vessel 1 with sampling tubes 13. Nutrient medium is passed through the vessel 1 upwardly, in the direction of the arrows, on operation of the pump P. Medium is taken from, and returned to, a sealed mixing reservoir 14, e.g. 1 litre in volume. The mixing reservoir 14, schematically represented in Fig. 6,



-5-

is illustrated in more detail in Fig. 7; it includes a fresh medium inlet 15, a medium collection outlet 16, a culture vessel medium outlet 17, a spent culture vessel medium inlet 18, an air/CO<sub>2</sub> inlet 19, an air outlet 20, a sample collection tube 21, and a pH probe 22. Stirring can be effected by means of a magnetic stirrer 23 and magnetic follower 24 (shown only in Fig. 7).

Fig. 6 also shows a fresh medium reservoir 9, a pH meter/controller 25 and, electrically connected thereto, a pump 26 which introduces fresh medium, when necessary, and removes medium for collection (see also Example 4). The use of apparatus as illustrated in Figs. 6 and 7 allows maximum economy in terms of medium usage, and also the recirculation of products of cell metabolism, lysis, etc. which may beneficially aid cell growth.

The following Examples illustrate the invention.

#### Example 1

An apparatus was constructed in borosilicate glass, with the properties and dimensions shown in Fig. 2. The glass was cleaned with chromic acid, and then attached by silicon tubing to a reservoir and filling bell, as shown in Fig. 3. The entire apparatus was flushed through with a concentrated solution of citric acid and then with at least 10 changes of double glass-distilled water. The entire apparatus was sterilised by autoclaving.

Growth medium (RPMI 1640 with 10% selected foetal calf serum) was placed in the reservoir, and run through the apparatus. The flow rate was controlled by a variable tubing clamp and measured by collecting medium in a measuring cylinder below the filling bell for measured times. Cells (see Examples 2 and 3) were introduced into the tubing with an hypodermic needle and syringe. At a flow rate of 0.5 ml/min, the cells formed horizontal bands in the apparatus. The entire apparatus

35

-6-

was mounted in a frame, and placed in a carbon dioxide incubator at 37 C, pH 100%, 95% carbon dioxide. The medium reservoir was replenished with medium as necessary, and the effluent medium was collected in  
5 sterile glass containers.

The medium collected from the filling bell was tested for immunoglobulin and antibody activity. The immunoglobulin levels were about 1 to 5 µg/ml, and antibody activity was detectable by a sensitive enzyme  
10 immuno-assay.

#### Example 2

Human lymphoblastoid cells were introduced into apparatus as in Example 1.

At a flow rate of 0.5 ml/min, a band of single cells  
15 could be seen several centimetres from the bottom of the apparatus. Clumps of cells, of various sizes, were present below the single cells, the larger clumps being nearer the bottom. A large "mass" of cells or clumps was present in the tube at the bottom of the apparatus; as  
20 the flow rate was increased each "band" of cells and clumps moved upward in the apparatus, when it became clear that the large "mass" at the bottom consisted of larger clumps of cells.

The human lymphoblastoid cells produced antibody  
25 against Pseudomonas aeruginosa serotype 11.

The culture was maintained for 35 days. During this time the number of cells appeared to increase (the bands became denser, and the lowermost aggregate of clumps became larger). The concentration of human  
30 immunoglobulin in the collected medium remained at between 1 and 5 µg/ml.

After 35 days, the culture became contaminated with bacteria.

-7-

Example 3

Hybrid cells, derived from the fusion of mouse myeloma NS0 and mouse spleen cells producing antibody, were introduced into apparatus as in Example 1.

- 5       The cells did not aggregate. They formed a band less than one centimetre deep, several centimetres above the bottom of the apparatus.

10       The culture was maintained for 28 days. During this time the density of the band varied. The concentration of mouse immunoglobulin in the collected medium remained at between 3 and 5 µg/ml.

After 28 days, the culture became contaminated with bacteria.

Example 4

- 15       The culture vessel illustrated in Fig. 5 was incorporated into a fluid flow circuit, as illustrated in Fig. 6. The culture vessel was constructed from a two litre volumetric flask. Medium was pumped into the culture at a controlled rate using a Watson Marlow pump,  
20       type MHRE 200. The medium as pumped from, and then back into, a reservoir, which was a Gallenkamp Culture Vessel Module (catalogue number FBL-195-010D), with magnetic stirrer (FBL-280) and magnetic follower, rotating at 60 rpm. The reservoir was supplied with 10% carbon dioxide  
25       in air at 200 ml/min, as illustrated in more detail in Fig. 7. The ports in the top plate of the reservoir carried a filtered air outlet, with reflux air condenser, a pH probe attached to a pH meter (FBL-734-010D), a medium inlet tube, a medium outlet tube, a sampling tube,  
30       and a carbon dioxide and air inlet tube. All glass tubing was light-wall borosilicate glass, outside diameter 4 mm, wall thickness 0.8 mm. All flexible tubing was medical grade silicone rubber, internal diameter 3 mm, outside diameter 6 mm; before use it was  
35       boiled in double-distilled water for one hour and stored

-8-

in distilled water. The pH meter was modified (by Gallenkamp) to drive a two-channel peristaltic pump for the medium inlet and the medium outlet. As the pH dropped to 7.0, fresh medium was introduced and used  
5 medium removed, to restore the pH to 7.3. The assembled apparatus and tubing were autoclaved at 100 kPa (15 psi) for 15 minutes.

The culture vessel was maintained at 37 C and the reservoir was maintained at 4 C. The two were connected  
10 by coils of borosilicate glass tubing; the temperature of the medium was raised or lowered by passage from one to the other. The apparatus and tubing were filled with RPMI1640 medium supplemented with 10% foetal calf serum, L-glutamine and sodium pyruvate, but without antibiotics.

15 Hybrid cells, derived from the fusion of a mouse myeloma cell with a mouse splenic B lymphocyte, were introduced into the culture vessel. They formed a band, the height of which was dependent on the flow rate of the medium.

20 During the culture period of 21 days, the concentration of hybrid cells in the band rose from  $3 \times 10^7$  per ml to  $8 \times 10^{12}$  per ml. The band of cells was clearly visible. Mouse immunoglobulin, with specific antibody, was harvested from the apparatus over a period  
25 of 14 days. At the end of this time, a contaminated batch of medium caused bacterial contamination of the entire culture.

30

35

-9-

CLAIMS

1. A method for the growth of cells in a nutrient medium, which comprises causing the nutrient medium to flow upwardly with decreasing velocity, substantially  
5 without turbulent flow, and allowing cells to grow at an equilibrium level in the flow.
2. A method according to claim 1, which comprises introducing medium at the lower end of a vessel having increasing and then decreasing cross-section, in the  
10 direction of flow, and removing medium together with any products of cell metabolism from the upper end of the vessel.
3. A method according to claim 1 or claim 2, in which the medium is regenerated after use and recirculated.
- 15 4. Apparatus which comprises:  
a vessel through which a nutrient medium can be passed upwardly, having increasing and then decreasing cross-section, in the direction of flow;  
means for regenerating the medium; and  
20 means for circulating the medium through the vessel and the regeneration means.
5. Apparatus according to claim 4, which additionally comprises:  
means for monitoring the condition of the medium, in  
25 response to which fresh medium can be introduced.

30

35

1/5

Fig.1.

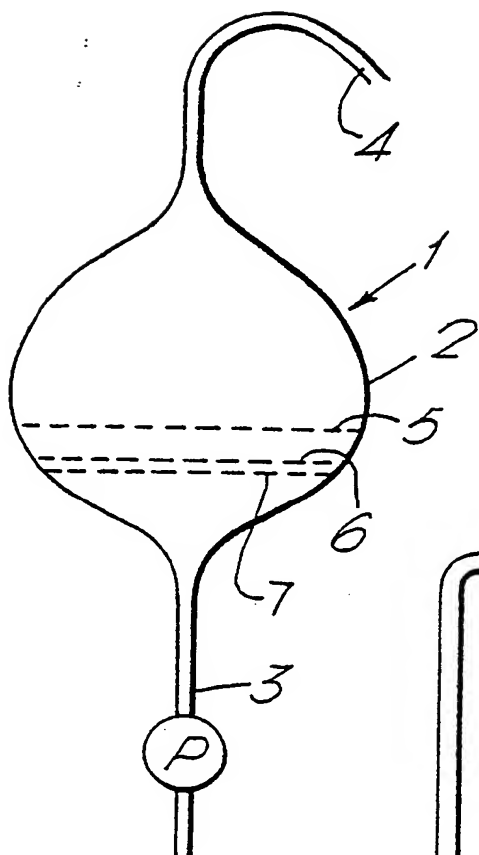
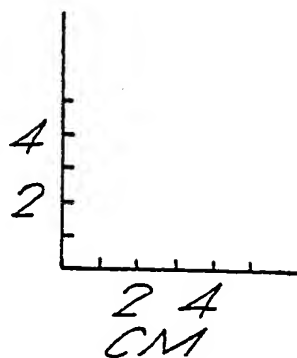
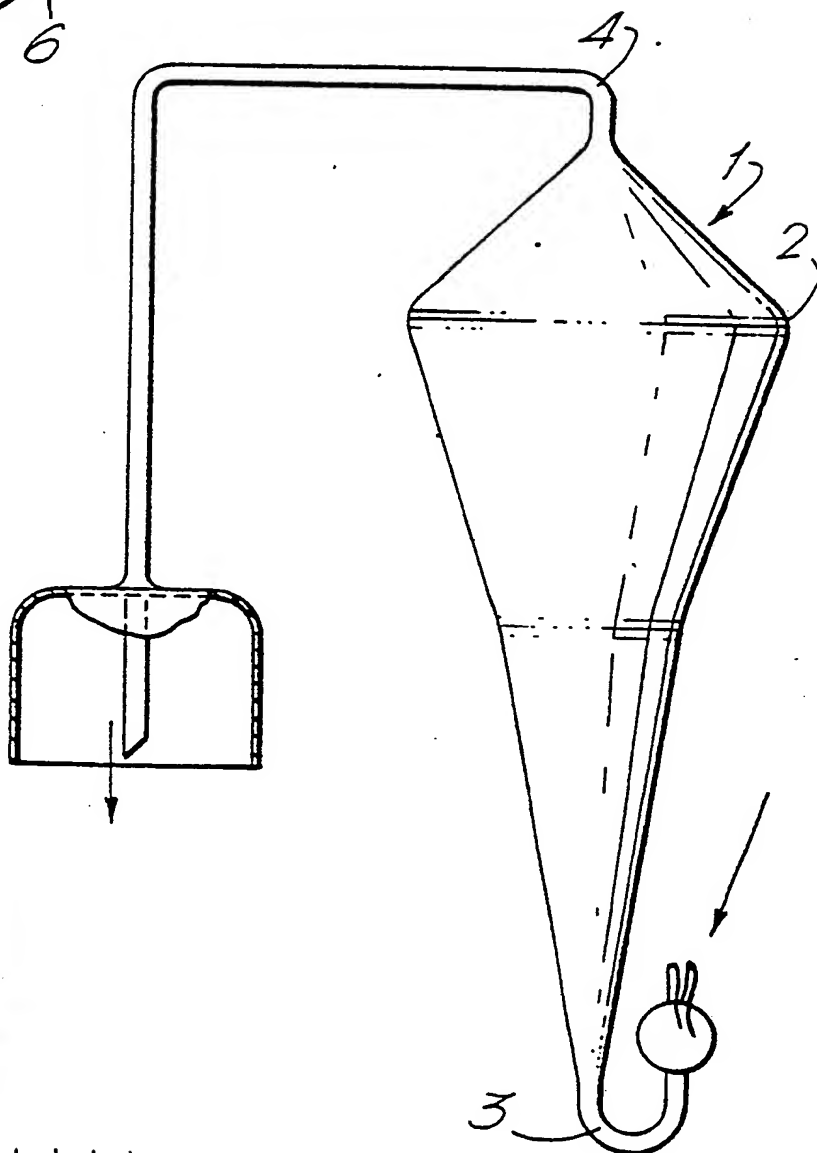
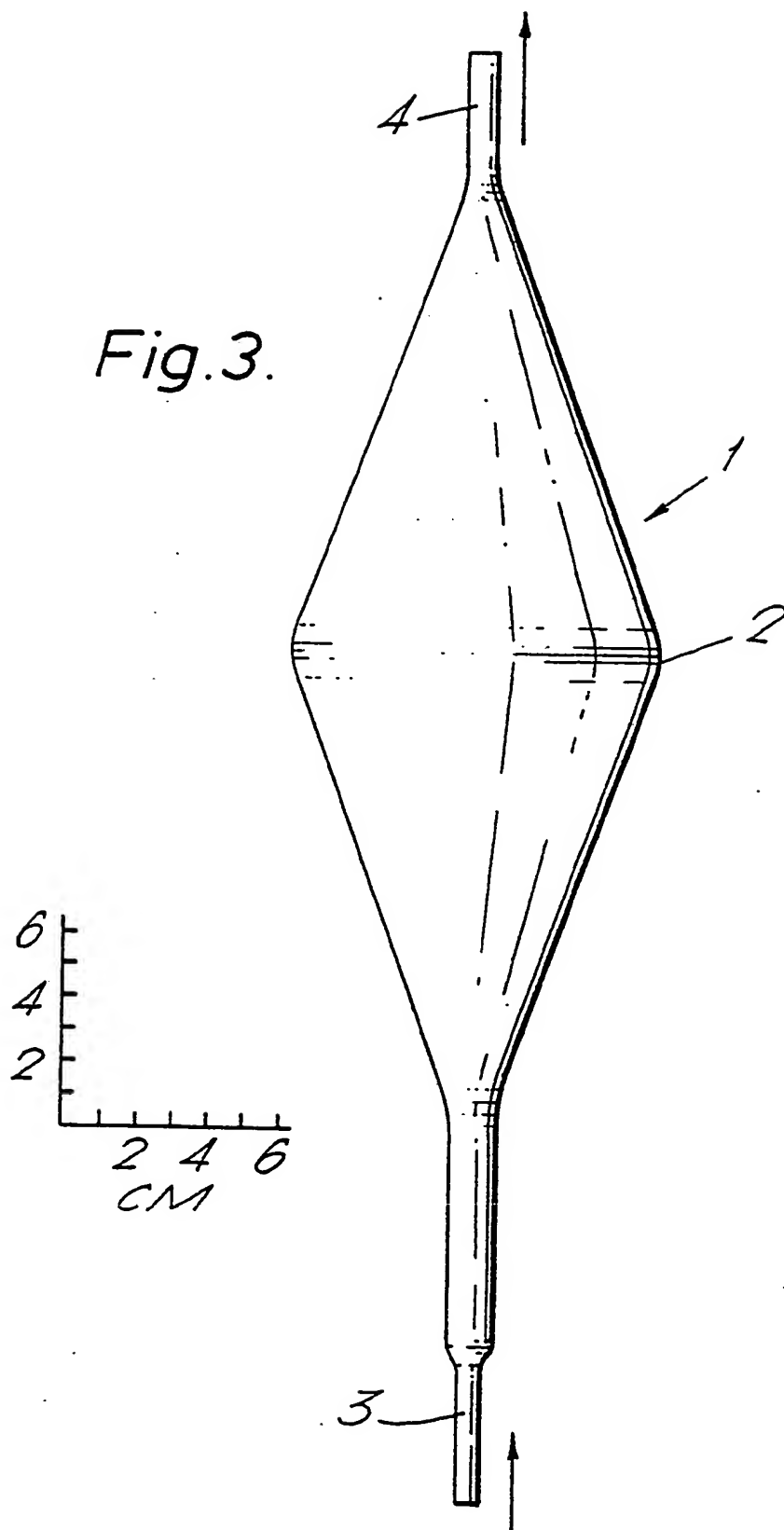


Fig.2.



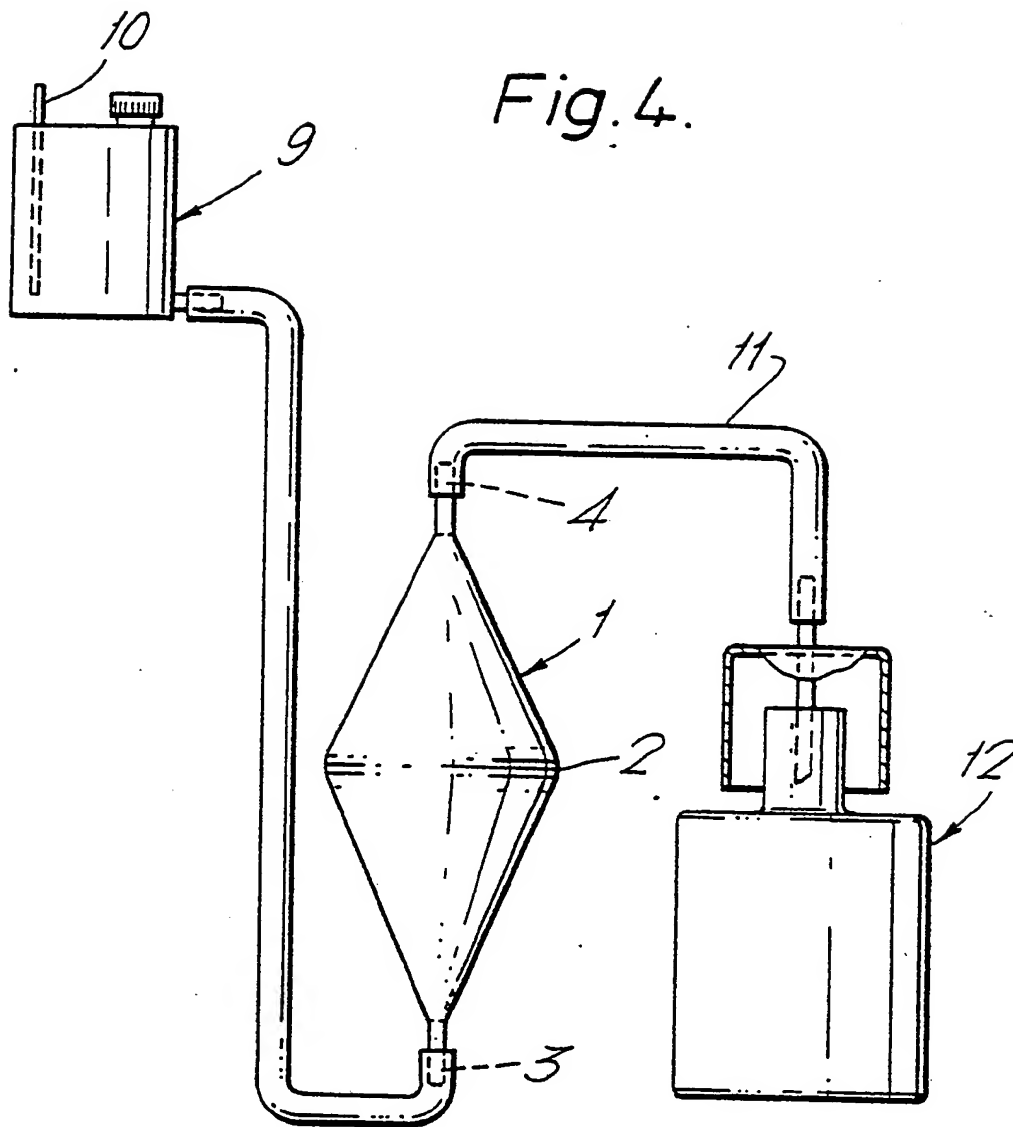
215

Fig.3.



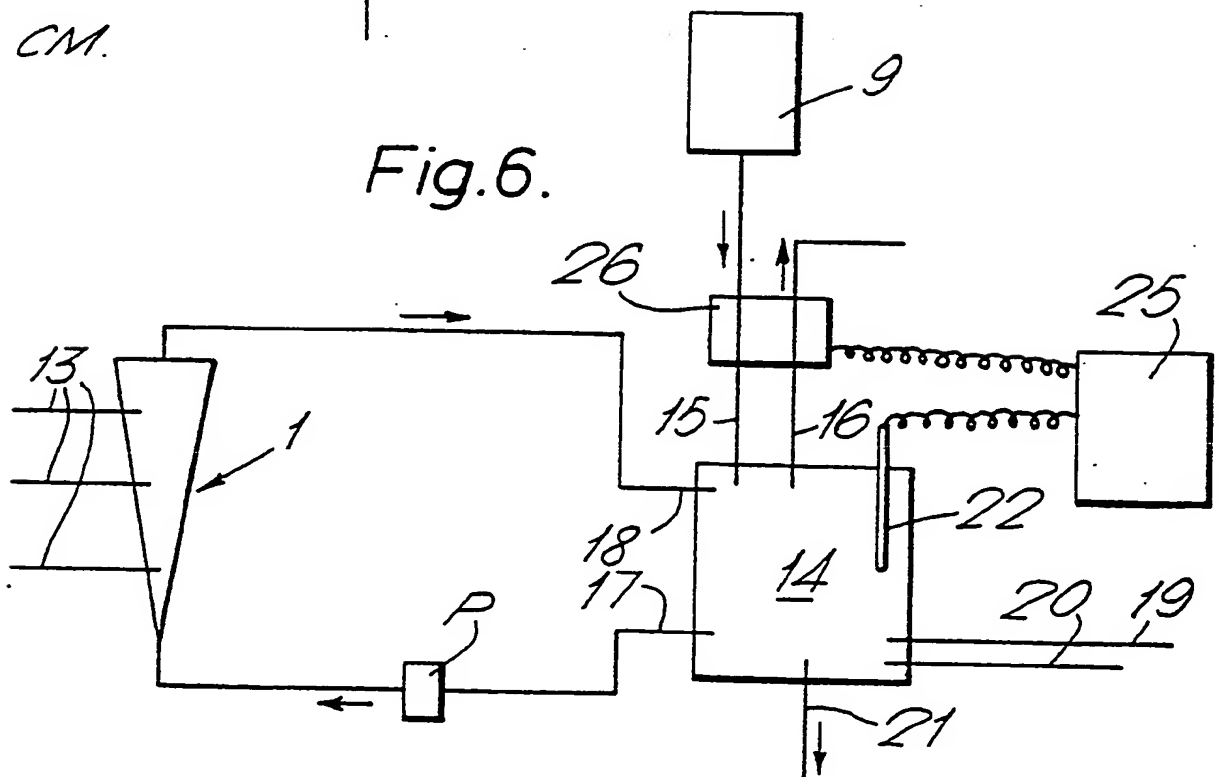
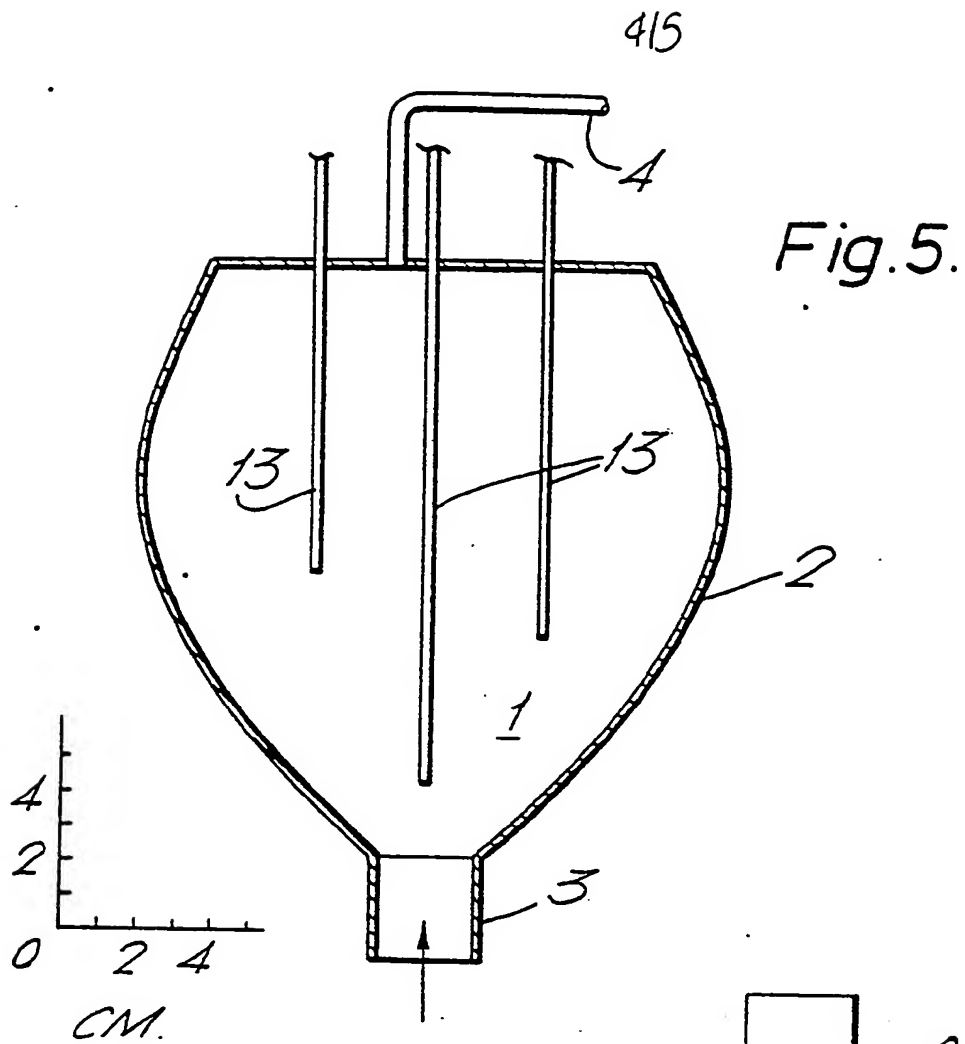
3/5

Fig. 4.



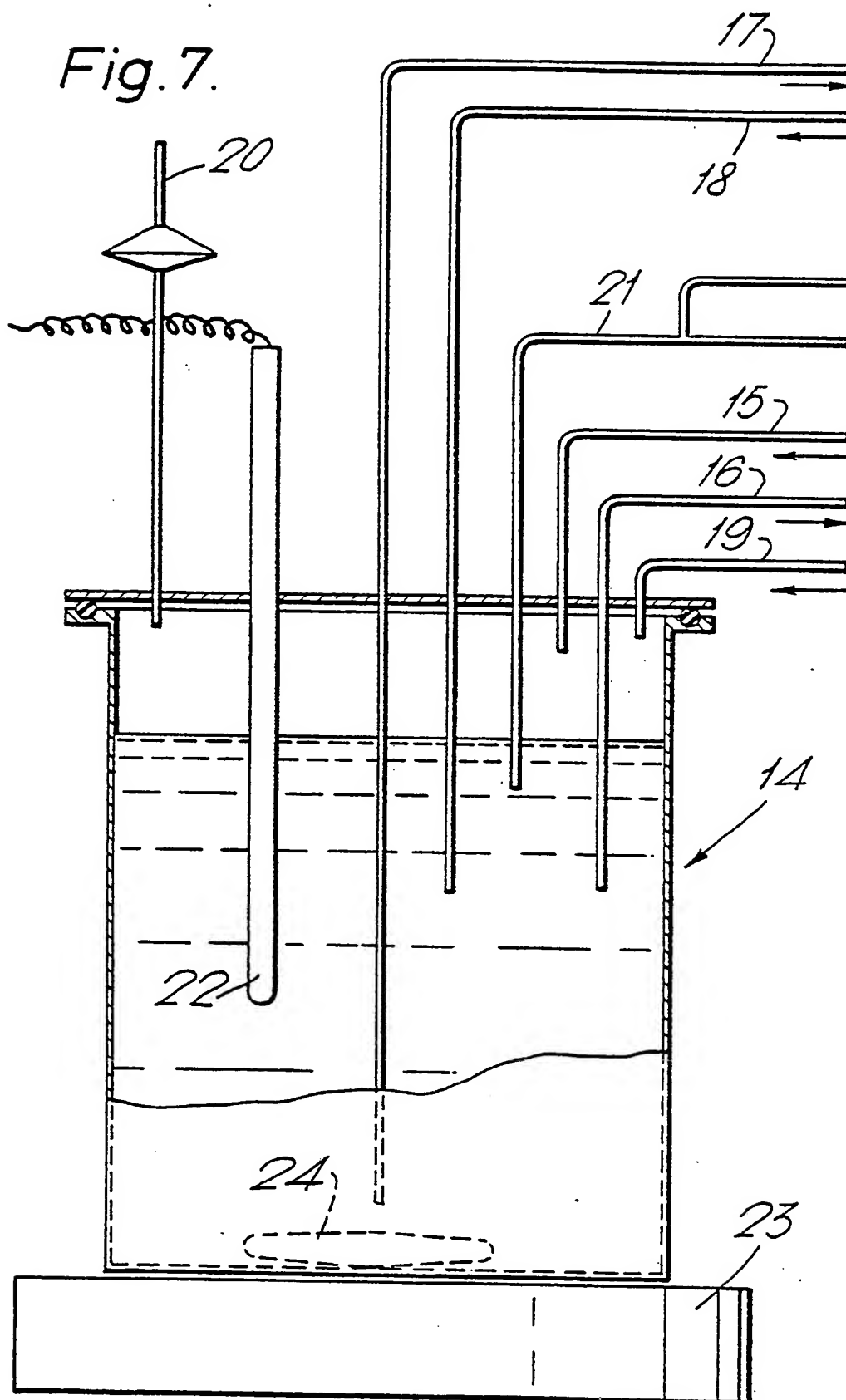
SUBSTITUTE SHEET





5/5

Fig. 7.



# INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 85/00307

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (if several classification symbols apply, indicate all) <sup>4</sup> According to International Patent Classification (IPC) or to both National Classification and IPC IPC <sup>4</sup> : C 12 M 3/02										
<b>II. FIELDS SEARCHED</b> <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black; margin: 5px 0;">Minimum Documentation Searched <sup>7</sup></div> <table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 30%; border-bottom: 1px solid black; padding: 5px;">Classification System  </td> <td style="border-bottom: 1px solid black; padding: 5px;">Classification Symbols</td> </tr> <tr> <td style="border: 1px solid black; padding: 10px; vertical-align: top;">IPC<sup>4</sup></td> <td style="border: 1px solid black; padding: 10px; vertical-align: top;">C 12 M</td> </tr> </table> <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black; margin: 5px 0;">Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched <sup>8</sup></div>			Classification System	Classification Symbols	IPC <sup>4</sup>	C 12 M				
Classification System	Classification Symbols									
IPC <sup>4</sup>	C 12 M									
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT <sup>9</sup></b> <table style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 10%; border-bottom: 1px solid black; padding: 5px;">Category <sup>9</sup></th> <th style="width: 70%; border-bottom: 1px solid black; padding: 5px;">Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup></th> <th style="width: 20%; border-bottom: 1px solid black; padding: 5px;">Relevant to Claim No. <sup>13</sup></th> </tr> <tr> <td style="border: 1px solid black; padding: 10px; vertical-align: top; text-align: center;">X</td> <td style="border: 1px solid black; padding: 10px; vertical-align: top;">US, A, 3591460 (D.F. SMITH) 6 July 1971, see figures; claims; column 2, lines 15-67  <div style="text-align: center; margin-top: 20px;">-----</div></td> <td style="border: 1px solid black; padding: 10px; vertical-align: top; text-align: center;">1-5</td> </tr> </table>			Category <sup>9</sup>	Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No. <sup>13</sup>	X	US, A, 3591460 (D.F. SMITH) 6 July 1971, see figures; claims; column 2, lines 15-67  <div style="text-align: center; margin-top: 20px;">-----</div>	1-5		
Category <sup>9</sup>	Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No. <sup>13</sup>								
X	US, A, 3591460 (D.F. SMITH) 6 July 1971, see figures; claims; column 2, lines 15-67  <div style="text-align: center; margin-top: 20px;">-----</div>	1-5								
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p><sup>10</sup> Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"Δ" document member of the same patent family</p> </div> </div>										
<b>IV. CERTIFICATION</b> <table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 50%; border-bottom: 1px solid black; padding: 5px;">Date of the Actual Completion of the International Search</td> <td style="width: 50%; border-bottom: 1px solid black; padding: 5px;">Date of Mailing of this International Search Report</td> </tr> <tr> <td style="border-bottom: 1px solid black; padding: 5px; text-align: center;">10th October 1985</td> <td style="border-bottom: 1px solid black; padding: 5px; text-align: center;">01 NOV 1985</td> </tr> <tr> <td style="border-bottom: 1px solid black; padding: 5px;">International Searching Authority</td> <td style="border-bottom: 1px solid black; padding: 5px;">Signature of Authorized Officer</td> </tr> <tr> <td style="border-bottom: 1px solid black; padding: 5px; text-align: center;">EUROPEAN PATENT OFFICE</td> <td style="border-bottom: 1px solid black; padding: 5px; text-align: center;">             G. M. Kruidenberg         </td> </tr> </table>			Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	10th October 1985	01 NOV 1985	International Searching Authority	Signature of Authorized Officer	EUROPEAN PATENT OFFICE	 G. M. Kruidenberg
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report									
10th October 1985	01 NOV 1985									
International Searching Authority	Signature of Authorized Officer									
EUROPEAN PATENT OFFICE	 G. M. Kruidenberg									

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON

INTERNATIONAL APPLICATION NO.

PCT/GB 85/00307 (SA 10086)

This Annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 23/10/85

The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US-A- 3591460	06/07/71	None	

For more details about this annex :  
see Official Journal of the European Patent Office, No. 12/82